CLAIM

1. A method of cell culture comprising

(i) culturing mammalian cells in a cell culture medium, and

(ii) maintaining at least one metabolite selected from aconitic acid (AA), leucinic acid (HICA), cytidine monophosphate (CMP), methylsuccinic acid (MSA), trigonelline (TRI) and N-acetylputrescinium (NAP) below an inhibitory concentration in the cell culture medium for the at least one metabolite, wherein:

the cell culture medium has a pH of 5.5 to 8.5;

the inhibitory concentration of AA is lower than 10 mM;

the inhibitory concentration of HICA is lower than 3 mM;

the inhibitory concentration of CMP is lower than 1 mM;

the inhibitory concentration of MSA is lower than 3 mM;

the inhibitory concentration of TRI is lower than 3 mM; and

the inhibitory concentration of NAP is lower than 3 mM.

2. The method of claim 1, wherein:

the at least one metabolite includes AA and the inhibitory concentration is lower than 880 μM,

the at least one metabolite includes HICA and the inhibitory concentration is lower than 47 μM,

the at least one metabolite includes CMP and the inhibitory concentration is lower than 20 μM,

the at least one metabolite includes MSA and the inhibitory concentration is lower than 7.5 μM,

the at least one metabolite includes TRI and the inhibitory concentration is lower than 0.7 μM,

the at least one metabolite includes NAP and the inhibitory concentration is lower than 0.6 μM, or combinations thereof.

3. The method of claim 1, wherein:

the at least one metabolite includes AA and the inhibitory concentration of AA is lower than 440 μM,

the at least one metabolite includes HICA and the inhibitory concentration of HICA is lower than 23.5 μM,

the at least one metabolite includes CMP and the inhibitory concentration of CMP is lower than 10 μM,

the at least one metabolite includes MSA and the inhibitory concentration of MSA is lower than 3.75 μM,

the at least one metabolite includes TRI and the inhibitory concentration of TRI is lower than 0.35 μM

the at least one metabolite includes NAP and the inhibitory concentration of NAP is lower than 0.3 μM, or

combinations thereof.

4. The method of claim 1, wherein the concentration of the at least one metabolite is measured using nuclear magnetic resonance (NMR) spectroscopy, Raman spectroscopy, High/Ultra Performance Liquid Chromatography (H/UPLC), Liquid Chromatography Mass Spectrometer (LC-MS), Gas Chromatography Mass Spectrometer (GC-MS) technology, or a combination thereof.

5. The method of claim 1, wherein step (ii) comprises measuring the concentration of the at least one metabolite, and, when the measured concentration of the at least one metabolite is above a predefined value, a concentration of a precursor of the at least one metabolite in the cell culture medium is decreased by reducing the amount of the precursor provided to the mammalian cells.

6. The method of claim 5, wherein

the at least one metabolite includes AA, and the precursor of AA is glutamine, glucose, arginine, asparagine, or a combination thereof;

the at least one metabolite includes HICA, and the precursor of HICA is leucine, isoleucine, or a combination thereof;

the at least one metabolite includes CMP, and the precursor of CMP is glutamine, arginine, aspartate, or a combination thereof;

the at least one metabolite includes MSA, and the precursor MSA is lysine, isoleucine, serine, glucose, glutamine, or a combination thereof;

the at least one metabolite includes TRI, and the precursor of TRI is aspartate, tryptophan, glutamine, or a combination thereof;

the at least one metabolite includes NAP, and the precursor of NAP is arginine, proline, aspartate glutamine, asparagine, or a combination thereof; or

a combination thereof.

7. The method of claim 1, wherein step (ii) comprises measuring the concentration of the at least one metabolite, and, when the measured concentration of the at least one metabolite is above a predefined value, regulating enzyme expression to reduce the synthesis of the metabolite.

8. The method of claim 7, wherein:

the at least one metabolite includes AA and the enzyme to reduce the synthesis of AA is acireductone dioxygenase 1 (ADI1), 4-hydroxy-2-oxoglutarate aldolase 1 (HOGA1), trans-aconitate decarboxylase (TAD1), or a combination thereof;

the at least one metabolite includes HICA and the enzyme to reduce the synthesis of HICA is glutamic-oxaloacetic transaminase 1 (GOT1), D-2-hydroxyisocaproate dehydrogenase (D-HicDH), methylmalonyl-CoA mutase (MMUT), 3-methylglutaconyl-CoA hydratase (AUH), 3-hydroxy-3-methylglutaryl-CoA lyase (HMGCL), hydroxyacyl-CoA dehydrogenase trifunctional multienzyme complex subunit alpha/beta (HADHA/B), or a combination thereof;

the at least one metabolite includes CMP and the enzyme to reduce the synthesis of CMP is uridine-cytidine kinase 1/2 (UCK1/2), 5'-nucleotidase ecto (NT5), cytidine monophosphate N-acetylneuraminic acid synthetase (CMAS), cytidine/uridine monophosphate kinase 1 (CMPK1), cytidine deaminase (CDA), solute carrier family 35 member A1 (SLC35A1), ribonucleotide reductase catalytic subunit M1 (RRM1), 4-hydroxy-2-oxoglutarate aldolase 1 (HOGA1), or a combination thereof;

the at least one metabolite includes MSA and the enzyme to reduce the synthesis of MSA is GOT1, ethylmalonic encephalopathy protein 1 (ETHE1), aminomethyltransferase (AMT), HADHA/B, MMUT, or a combination thereof;

the at least one metabolite includes TRI and the enzyme to reduce the synthesis ofTRI is nicotinamide adenine dinucleotide synthetase 1 (NADSYN1), nicotinamide N-methyltransferase

(NNMT), catalase (CAT), nicotinamide nucleotide adenylyltransferase 1 (NMNAT1), sulfotransferase family 4A member 1 (SULT4A1), or a combination thereof;

the at least one metabolite includes NAP and the enzyme to reduce the synthesis of NAP is spermidine/spermine N1-acetyltransferase 1/2 (SAT1/2), HOGA1, adenosylmethionine decarboxylase 1 (AMD1), ornithine decarboxylase 1 (ODC1), GOT1, monoamine oxidase B (MAOB), or a combination thereof; or

a combination thereof.

9. The method of claim 7, wherein regulating enzyme expression to reduce the synthesis of the metabolite comprises adding an inhibitory nucleic acid which inhibits expression of a gene encoding the enzyme.

10. The method of claim 7, wherein regulating enzyme expression to reduce the synthesis of the metabolite comprises adding a recombinant DNA molecule to overexpress the regulating enzyme, wherein the regulating enzyme is a downstream enzyme.

11. The method of claim 1, wherein step (ii) comprises measuring the concentration of the at least one metabolite, and, when the measured concentration of the at least one metabolite is above a predefined value, regulating enzyme activity to reduce the synthesis of the metabolite.

12. The method of claim 11, wherein:

the at least one metabolite includes AA and the enzyme to reduce the synthesis of AA is acireductone dioxygenase 1 (ADI1), 4-hydroxy-2-oxoglutarate aldolase 1 (HOGA1), trans-aconitate decarboxylase (TAD1), or a combination thereof;

the at least one metabolite includes HICA and the enzyme to reduce the synthesis of HICA is glutamic-oxaloacetic transaminase 1 (GOT1), D-2-hydroxyisocaproate dehydrogenase (D-HicDH), methylmalonyl-CoA mutase (MMUT), 3-methylglutaconyl-CoA hydratase (AUH), 3-hydroxy-3-methylglutaryl-CoA lyase (HMGCL), hydroxyacyl-CoA dehydrogenase trifunctional multienzyme complex subunit alpha/beta (HADHA/B), or a combination thereof;

the at least one metabolite includes CMP and the enzyme to reduce the synthesis of CMP is uridine-cytidine kinase 1/2 (UCK1/2), 5'-nucleotidase ecto (NT5), cytidine monophosphate N-acetylneuraminic acid synthetase (CMAS), cytidine/uridine monophosphate kinase 1 (CMPK1), cytidine deaminase (CDA), solute carrier family 35 member A1 (SLC35A1), ribonucleotide reductase catalytic subunit M1 (RRM1), 4-hydroxy-2-oxoglutarate aldolase 1 (HOGA1), or a combination thereof;

the at least one metabolite includes MSA and the enzyme to reduce the synthesis of MSA is GOT1, ethylmalonic encephalopathy protein 1 (ETHE1), aminomethyltransferase (AMT), HADHA/B, MMUT, or a combination thereof;

the at least one metabolite includes TRI and the enzyme to reduce the synthesis of TRI is nicotinamide adenine dinucleotide synthetase 1 (NADSYN1), nicotinamide N-methyltransferase (NNMT), catalase (CAT), nicotinamide nucleotide adenylyltransferase 1 (NMNAT1), sulfotransferase family 4A member 1 (SULT4A1), or a combination thereof;

the at least one metabolite includes NAP and the enzyme to reduce the synthesis of NAP is spermidine/spermine N1-acetyltransferase 1/2 (SAT1/2), HOGA1, adenosylmethionine decarboxylase 1 (AMD1), ornithine decarboxylase 1 (ODC1), GOT1, monoamine oxidase B (MAOB), or a combination thereof; or

a combination thereof.

13. The method of claim 11, wherein regulating enzyme activity to reduce the synthesis of the at least one metabolite comprises adding an inhibitor of enzyme activity to the culture.

14. The method of claim 11, wherein regulating enzyme activity to reduce the synthesis of the at least one metabolite comprises adding a glycolytic pathway activator to the culture.

15. The method of claim 1, wherein maintaining the at least one metabolite below an inhibitory concentration in the cell culture medium for the at least one metabolite comprises controlling temperature, dissolved oxygen level, pH, or a combination thereof.

16. The method of any of the foregoing claims, wherein the mammalian cells are CHO cells, HEK 293 cells, HT-1080 cells, engineered T cells, or engineered natural killer cells.

17. The method of any of the foregoing claims, wherein the cell culture is a batch culture, a fed batch culture, or a perfusion culture.

18. The method of any of the foregoing claims, wherein the mammalian cells express a recombinant protein, a gene product, or a cell product.

19. The method of claim 18, further comprising obtaining and purifying the recombinant protein, gene product, or cell product.

20. The method of claim 18, wherein the recombinant protein is a monoclonal antibody.

21. The method of any of the foregoing claims, wherein cell growth and/or productivity are increased as compared to a control culture, wherein the control culture is identical to the culture of claim 1 except it is not cultured using step (ii).

22. The method of claim 21, wherein the cell growth is determined by maximum viable cell density and is increased by at least 5% as compared to the control culture.

23. The method of any of the foregoing claims, wherein the maximum viable cell density of the cell culture is greater than 1×10^6 cells/mL.

24. The method of any of the foregoing claims, wherein the cell culture method comprises a growth phase and a production phase and step (ii) is applied during the growth phase.